Verification of Translation

I, Shuji Yoshizaki, do hereby declare that I am familiar with Japanese and English Languages, and the attached document is a faithful English translation of Japanese Patent Application No. 2002-265982.

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[Attachments]

[Document] Specification 1

[Document] Drawing 1

[Document] Abstract 1

[Document] Certification of share 1

[Special note of the attachment] Supplemented later 1

[Proof]

Required.

[Name of Document] Specification

[Title of Invention] Biological law molecular weight derivatives

[Claims]

[Claim 1] A biological low-molecular-weight derivative obtained by modifying at least one carboxyl group of a biological low-molecular-weight compound having two or more carboxyl groups with N-hydroxysuccinimide, N-hydroxysulfosuccinimide, or a derivative thereof.

[Claim 2] The biological low-molecular-weight derivative according to claim 1, wherein the biological low-molecular-weight compound having two or more carboxyl groups is a low-molecular-weight compound in the citric acid cycle or a derivative thereof.

[Claim 3] The biological low-molecular-weight derivative according to claim 1, wherein the biological low-molecular-weight compound having two or more carboxyl groups is malic acid, oxalacetic acid, citric acid, *cis*-aconitic acid, 2-ketoglutaric acid, or a derivative thereof.

[Claim 4] A crosslinked high-molecular-weight product obtained by crosslinking a high-molecular-weight compound with the biological low-molecular-weight derivative according to claim 1.

[Claim 5] The crosslinked high-molecular-weight product according to claim 4, wherein the high-molecular-weight compound is at least one of proteins, glycosaminoglycans, chitosans, polyamino acids, and polyalcohols.

[Claim 6] The crosslinked high-molecular-weight product according to claim 4, wherein the high-molecular-weight compound is a glycosaminoglycan comprising chondroitin sulfate, dermatan sulfate, hyaluronic acid, heparan sulfate, heparin, keratan sulfate, or a derivative thereof.

[Claim 7] The crosslinked high-molecular-weight product according to claim 4, wherein the high-molecular-weight compound is a protein comprising collagen, atelocollagen, alkali-soluble collagen, gelatin, keratin, serum albumin, egg albumin, hemoglobin, casein, globulin, fibrinogen, or a derivative thereof.

[Detailed Description of Invention]

[0001]

[Technical Field]

The present invention relates to biological low-molecular-weight derivatives obtained by modifying carboxyl groups of biological low-molecular-weight compounds having two or more carboxyl groups with N-hydroxysuccinimide, N-hydroxysulfosuccinimide, or derivatives thereof, and to crosslinked high-molecular-weight products synthesized using the biological low-molecular-weight derivatives.

[0002]

[Background Art]

In biological adhesives and in treating medical devices that are derived from biological compounds, such as porcine cardiac valves, crosslinking agents containing artificially and chemically synthesized aldehydes such as glutaraldehyde or condensing agents such as 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide have been used (for example, refer to patent documents 1 to 6, and non-patent document 1).

[0003]

Patent document 1: Japanese Unexamined Patent Application Publication No. 7-163650

Patent document 2: Japanese Unexamined Patent Application Publication No. 9-249751

Patent document 3: Japanese Unexamined Patent Application

Publication No. 10-71199

Patent document 4: PCT Japanese Translation Patent Publication No. 2000-502380

Patent document 5: Japanese Unexamined Patent Application Publication No. 8-53548

Patent document 6: PCT Japanese Translation Patent Publication No. 8-502082

Non-patent document 1: Biomaterials, vol. 17, p. 765 (1996)

[0005]

[Disclosure of Invention]

Most crosslinking agents and condensing agents that have been used to treat medical devices and the like are non-natural, artificially synthesized products. Thus, they are not metabolized in vivo and exhibit toxicity to living bodies. They are thus used in limited amounts and for limited purposes in clinical sites. In order to overcome such problems, development of biological crosslinking agents is desired.

[0006]

[Means to solve the objectives]

The present invention provides a biological low-molecular-weight derivative obtained by modifying carboxyl groups of a biological low-molecular-weight compound having two or more carboxyl groups with N-hydroxysuccinimide, N-hydroxysulfosuccinimide, or a derivative thereof, and a

crosslinked high-molecular weight product synthesized using the biological low-molecular-weight derivative.

[0007]

In detail, the present invention provides a biological low-molecular-weight derivative obtained by modifying at least one carboxyl group of a biological low-molecular-weight compound having two or more carboxyl groups with N-hydroxysuccinimide, N-hydroxysulfosuccinimide, or a derivative thereof. This biological low-molecular-weight derivative is harmless to human bodies and achieves fast reaction since two or more reactive groups (-COOH) are contained.

[0008]

The present invention also provides a crosslinked high-molecular weight product prepared by crosslinking a high-molecular-weight compound with a biological low-molecular-weight derivative obtained by modifying at least one carboxyl group of a biological low-molecular-weight compound having two or more carboxyl groups with N-hydroxysuccinimide, N-hydroxysulfosuccinimide, or a derivative thereof.

When this crosslinked high-molecular weight product is applied to living bodies, the compound is metabolized in vivo, and is absorbed and disappears after a predetermined time. Thus, no extraneous matter remains in the body.

[0009]

[Embodiments of the Invention]

A biological low-molecular-weight compound having two or more carboxyl groups used in the present invention is a tri- or dicarboxylic acid low-molecular-weight compound in the citric acid cycle. Examples of the tri- or dicarboxylic acid low-molecular-weight compound include malic acid, oxalacetic acid, citric acid, *cis*-aconitic acid, 2-ketoglutaric acid, and derivatives thereof.

[0010]

The biological low-molecular-weight derivative of the present invention is obtained by reacting carboxyl groups of the biological low-molecular-weight compound having two or more carboxyl groups with N-hydroxysuccinimide, N-hydroxysulfosuccinimide, or a derivative thereof having low cytotoxicity in the presence of carbodiimide to introduce active esters.

[0011]

Such a compound is obtained by reacting 0.001 to 10 percent by weight of the biological low-molecular-weight compound with 0.001 to 10 percent by weight of N-hydroxysuccinimide, N-hydroxysulfosuccinimide, or a derivative thereof in the presence of 0.001 to 20 percent by weight of carbodiimide (EDC) at a suitable reaction temperature in the range of 0°C to 100°C and a reaction time in the range of 1 to 48 hours.

[0012]

Examples of the carbodiimide include 1-ehtyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC), 1-cyclohexyl-3-(2-morpholinoethyl)carbodiimide metho-p-toluenesulfonate, and dicyclohexylcarbodiimide. Examples of the reaction solvent include N,N-dimethylformamide (DMF) and dimethylsulfoxide (DMSO).

[0013]

Fig. 1 is a chart showing a nuclear magnetic resonance spectrum of a citric acid derivative yielded by the reaction between citric acid and N-hydroxysuccinimide in the presence of EDC. The peak a shows the methylene proton of citric acid and the peak b shows the methylene proton of the succinimidyl group. The remaining two peaks are attributable to the solvent (DMSO).

[0014]

Examples of the protein used in preparing the crosslinked compound include collagen (any of several ten types), atelocollagen (any one of several ten types), alkali-soluble collagen (any of several ten types), gelatin, keratin, serum albumin, egg albumin, hemoglobin, casein, and amino-containing polymers such as globulin and fibrinogen. These proteins may be derived from any organism.

[0015]

Examples of glycosaminoglycans used to prepare the crosslinked product include chondroitin sulfate, dermatan

sulfate, hyaluronic acid, heparan sulfate, heparin, keratan sulfate, and their derivatives. The glycosaminoglycans may have any molecular weight and may be derived from any organism.

[0016]

Examples of other high-molecular-weight compounds include chitosan (the degree of deacetylation and molecular weight are not limited), polyamino acids (the type of amino acid and molecular weight are not limited), and polyalcohols (the type and molecular weight are not limited).

[0017]

The crosslinking reaction between the biological low-molecular-weight derivative and the high-molecular-weight compound is conducted by reacting 0.1 to 50 percent by weight of the high-molecular-weight compound with 0.01 to 50 percent by weight of the biological low-molecular-weight derivative at preferably 30°C to 50°C. These two compounds are preferably mixed as solutions having predetermined concentrations to facilitate synthesis of a homogeneous crosslinked product. Examples of the solvents used to prepare such solutions include nontoxic solvents such as distilled water, buffer solutions, e.g., physiological saline, sodium hydrogen carbonate, boric acid, and phosphoric acid, and organic solvents (DMF, DMSO, and ethanol).

[0018]

The gelate biological high-molecular-weight product

described above is applied to one of biological adhesives, hemostatic agents, materials for embolizing blood vessels, and sealing materials for aneurysum to perform crosslinking reaction directly at affected sites. The compound may be crosslinked in advance and then be applied to adhesion preventing agents, scaffolds for tissue regeneration, and drug carriers.

[0019]

(Examples)

Example 1-1

To a 5 wt% DMF solution of citric acid, 3.2 equivalents of N-hydroxysuccinimide and 3.1 equivalents of EDC were added at room temperature, and the resulting mixture was stirred for 24 hours. Subsequently, only the DMF, i.e., the organic solvent in the reaction solution, was removed by reduced-pressure distillation. The residue was purified with an acetone/n-hexane developing solvent by chromatography over a silica gel column to synthesize a derivative having three carboxyl groups of citric acid modified with N-hydroxysuccinimide.

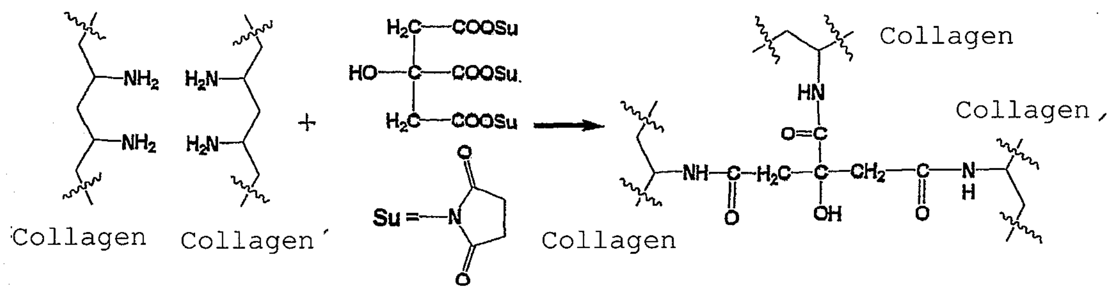
[0020]

Example 1-2

The synthesis of collagen gel obtained by the reaction scheme below using the citric acid derivative synthesized in Example 1-1 will now be described:

[0021]

(Chem. 1)



Citric acid derivative

[0022]

The synthesized citric acid derivative (24 μL) was dissolved in 976 μL of a dimethylsulfoxide solution. A 100 μL portion of this solution was weighed and added to 400 μL of a 1.25 wt% phosphoric acid buffer solution of type II collagen. The resulting mixture was stirred and left to stand still for 24 hours at room temperature to obtain a collagen gel having a crosslinking agent concentration of 0.4 to 40 mM. The gel was weighed, dried in a freeze-dry machine, and weighed again to determine the water content of the gel. The water content of the gel is shown in Table 1.

[0023]

(Table 1)

Concentration of citric acid derivative (mM)	Water content (%)
0.4	98
1	98
2	96
4	97
8	97
10	97
20	98
30	98
40	98

[0024]

Example 2-1

To a 5 wt% DMF solution of 2-ketoglutaric acid, 2.2 equivalents of N-hydroxysuccinimide and 2.1 equivalents of EDC were added at room temperature. The resulting mixture was stirred for 24 hours. Subsequently, only the DMF, i.e., the organic solvent in the reaction solution, was removed by reduced-pressure distillation. The residue was purified with an acetone/n-hexane developing solvent by chromatography over a silica gel column to obtain a derivative having two carboxyl groups of 2-ketoglutaric acid modified with N-hydroxysuccinimide.

[0025]

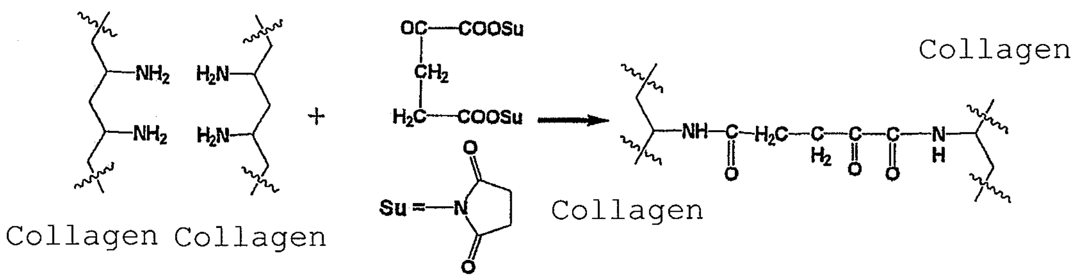
Example 2-2

The synthesis of collagen gel obtained by the reaction

scheme below using the 2-ketoglutaric acid derivative synthesized in Example 2-1 will now be described:

[0026]

(Chem. 2)



2-Ketoglutaric acid derivative

[0027]

The synthesized 2-ketoglutaric acid derivative (14 μL) was dissolved in 986 μL of a dimethylsulfoxide solution. A 100 μL portion of this solution was weighed and added to 400 μL of a 1.25 wt% phosphoric acid buffer solution of type II collagen. The resulting mixture was stirred and left to stand still for 24 hours at room temperature to obtain a collagen gel having a crosslinking agent concentration of 0.6 to 10 mM. The gel was weighed, dried in a freeze-dry machine, and weighed again to determine the water content of the gel. The water content of the gel is shown in Table 2.

[0028]

(Table 2)

Concentration of 2-ketogluraic acid derivative (mM)	Water content (%)
0.6	98
0.8	98
1	98
4	97
8	98
10	97

[0029]

Example 3-1

To a 5 wt% DMF solution of *cis*-aconitic acid, 3.2 equivalents of N-hydroxysuccinimide and 3.1 equivalents of EDC were added at room temperature. The resulting mixture was stirred for 24 hours. Subsequently, only the DMF, i.e., the organic solvent in the reaction solution, was removed by reduced-pressure distillation. The residue was purified with an acetone/n-hexane developing solvent by chromatography over a silica gel column to obtain a derivative having three carboxyl groups of *cis*-aconitic acid modified with N-hydroxysuccinimide.

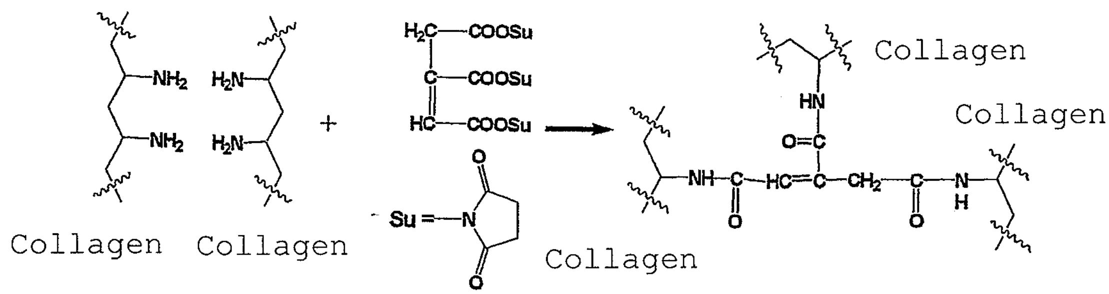
[0030]

Example 3-2

The synthesis of collagen gel obtained by the reaction scheme below using the *cis*-aconitic acid derivative synthesized in Example 3-1 will now be described:

[0031]

(Chem. 3)



cis-Aconitic acid derivative

[0032]

The synthesized *cis*-aconitic acid derivative (46 μL) was dissolved in 954 μL of a dimethylsulfoxide solution. A 100 μL portion of this solution was weighed and added to 400 μL of a 1.25 wt% phosphoric acid buffer solution of type II collagen. The resulting mixture was stirred and left to stand still for 24 hours at room temperature to obtain a collagen gel having a crosslinking agent concentration of 1 to 30 mM. The gel was weighed, dried in a freeze-dry machine, and weighed again to determine the water content of the gel. The water content of the gel is shown in Table 3.

[0033]

(Table 3)

Concentration of *cis*-aconitic acid derivative (mM)	Water content (%)
1	97
2	97
4	97
8	97
10	97
30	97

[0034]

Example 4-1

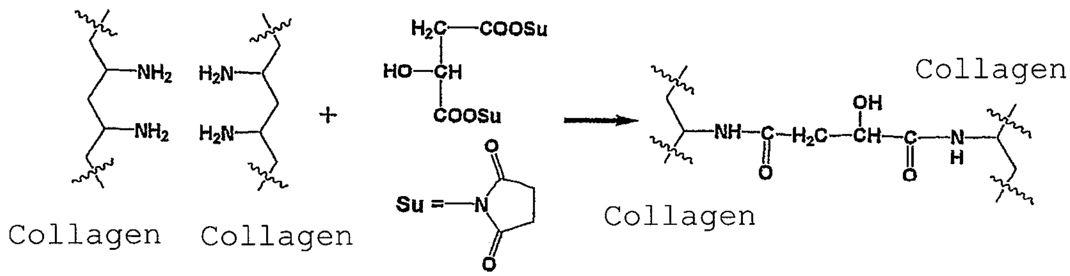
To a 5 wt% DMF solution of malic acid, 2.2 equivalents of N-hydroxysuccinimide and 2.1 equivalents of EDC were added at room temperature. The resulting mixture was stirred for 24 hours. Subsequently, only the DMF, i.e., the organic solvent in the reaction solution, was removed by reduced-pressure distillation. The residue was purified with an acetone/n-hexane developing solvent by chromatography over a silica gel column to obtain a derivative having two carboxyl groups of malic acid modified with N-hydroxysuccinimide.

[0035]

The synthesis of collagen gel obtained by the reaction scheme below using the malic acid derivative synthesized in Example 4-1 will now be described:

[0036]

(Chem. 4)



Malic acid derivative

[0037]

The synthesized malic acid derivative (32 μL) was dissolved in 968 μL of a dimethylsulfoxide solution. A 100 μL portion of this solution was weighed and added to 400 μL of a 1.25 wt% phosphoric acid buffer solution of type II collagen. The resulting mixture was stirred and left to stand still for 24 hours at room temperature to obtain a collagen gel having a crosslinking agent concentration of 3 to 50 mM. The gel was weighed, dried in a freeze-dry machine, and weighed again to determine the water content of the gel. The water content of the gel is shown in Table 4.

[0038]

(Table 4)

Concentration of malic acid derivative (mM)	Water content (%)
3	97
4	98
5	97
6	97
8	97
10	97
20	97
40	97
50	97

[0039]

Example 5-1

To a 5 wt% DMF solution of oxalacetic acid, 2.2 equivalents of N-hydroxysuccinimide and 2.1 equivalents of EDC were added at room temperature. The resulting mixture was stirred for 24 hours. Subsequently, only the DMF, i.e., the organic solvent in the reaction solution, was removed by reduced-pressure distillation. The residue was purified with an acetone/n-hexane developing solvent by chromatography over a silica gel column to obtain a derivative having two carboxyl groups of oxalacetic acid modified with N-hydroxysuccinimide.

[0040]

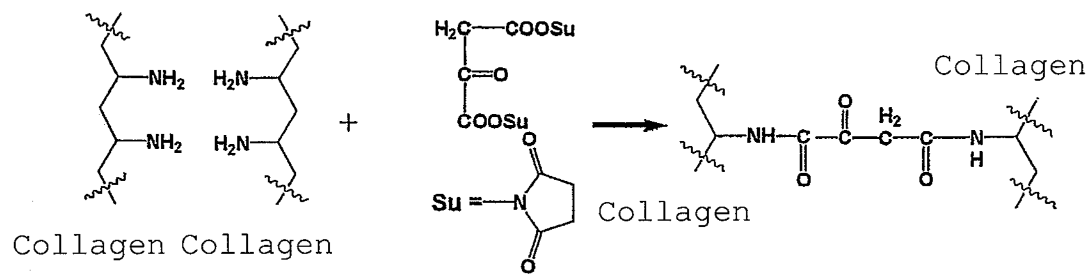
Example 5-2

The synthesis of collagen gel obtained by the reaction scheme below using the oxalacetic acid derivative synthesized

in Example 5-1 will now be described:

[0041]

(Chem. 5)



Oxalacetic acid derivative

[0042]

The synthesized oxalacetic acid derivative (16 μL) was dissolved in 984 μL of a dimethylsulfoxide solution. A 100 μL portion of this solution was weighed and added to 400 μL of a 1.25 wt% phosphoric acid buffer solution of type II collagen. The resulting mixture was stirred and left to stand still for 24 hours at room temperature to obtain a collagen gel having a crosslinking agent concentration of 2 to 40 mM. The gel was weighed, dried in a freeze-dry machine, and weighed again to determined the water content of the gel. The water content of the gel is shown in Table 5.

[0043]

(Table 5)

Concentration of oxalacetic acid derivative (mM)	Water content (%)
2	98
4	97
6	98
8	97
10	98
20	96
40	98

[Brief Description of the Drawing]

[Fig. 1] Fig. 1 is a chart showing a nuclear magnetic resonance spectrum of a citric acid derivative.